

## **TM LEVELING METHOD**

### **ABSTRACT OF THE DISCLOSURE**

Methods for designing an oligonucleotide sequence having a selected duplex stability are provided wherein the oligonucleotide portion comprises at least one modified base selected from universal bases, unsubstituted and 3-substituted pyrazolo[3,4-d]pyrimidines and 5-substituted pyrimidines, and optionally having attached minor groove binders and reporter groups.